APPLICATION NOTE

No. COL01848

Thermoinducible expression of DNA polymerase I

Comparison of single-use versus stainless steel systems for microbial fermentation

Introduction

Thermo Scientific™ HyPerforma™ Single-Use Fermentor (S.U.F.) is a unique, robust, and purpose-built solution for microbial fermentation applications, offering flexibility, ease-of-use, and efficiency found in single-use systems. This comparison of the HyPerforma S.U.F.s versus stainless steel fermentors demonstrates that the product yield and catalytic activity of the expressed enzyme were not significantly different.

Goal

In this study, the performance of thermoinducible (42°C) recombinant expression of DNA polymerase I from an *Escherichia coli (E. coli)* culture was compared between the S.U.F. and stainless steel fermentor systems.

Methods and materials

The thermoinducible expression system is based on the λphage gene expression controlling promoters *pL* which is regulated by the thermolabile mutant repressor *cI857* [1].

Four different systems were evaluated for this study: 10 L and 100 L stainless steel fermentors using heat exchangers, and 30 L and 300 L S.U.F.s using temperature control units (TCUs).

Each system underwent a preinduction, induction, and postinduction phase of the recombinant *E. coli* culture (Table 1). The induction point was set at an OD_{600} of about 0.5.

Table 1. Temperature conditions per phase.

Phase	Parameters
Preinduction	32°C until 0.5 OD_{600} is reached
Induction	Heated to 42°C
Postinduction	37°C for 3 hours

In all systems, 80% of the nominal volume was used with lysogeny broth medium. All dynamic fermentation parameters were programmed to maintain dissolved oxygen (DO) at 30%; pH at 7.0; and temperatures as indicated by Table 1.

Samples for protein analysis were harvested 10 minutes before the induction of the recombinant DNA polymerase I synthesis and 3 hours postinduction.

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For protein expression analysis, biomass samples were lysed using Thermo Scientific™ B-PER™ Complete Bacterial Protein Extraction Reagent; soluble and insoluble protein fractions were then separated by centrifugation.

Total, soluble, and insoluble protein fractions were visualized by SDS-PAGE. The activity of DNA polymerase I in the soluble fraction was estimated to determine whether it was correctly folded and functional.

The DNA polymerase I activity unit corresponds to the amount of enzyme that catalyzes incorporation of 10 nmol [^{3}H]-dTTP to the polynucleotide in 30 minutes at 37°C.

Both test samples and control were diluted (500X) and run in triplicates. Activity of the DNA polymerase I was measured in the following reaction mixture: DNA polymerase I buffer (1X); dNTP mix (0.033 mM); [^{3}H]-TTP (0.4 MBq/mL); and salmon sperm DNA (0.0625 mg/mL).

After the reaction, each mixture was embedded on a positively charged nylon membrane, washed three times with 7.5% Na_2HPO_4, and one time with water. The incorporation of radioactive nucleotides was measured in a scintillator [2].

Results

Thermoinduction culture plots

Temperature and OD_{600} are captured on the four systems in Figures 1–4. The pH was maintained at 7 for each system throughout the culture.

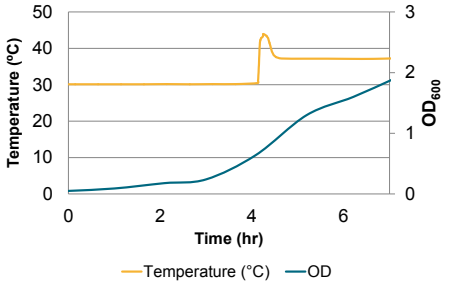

Figure 1. 100 L stainless steel fermentor: *E. coli* growth and culture temperature.

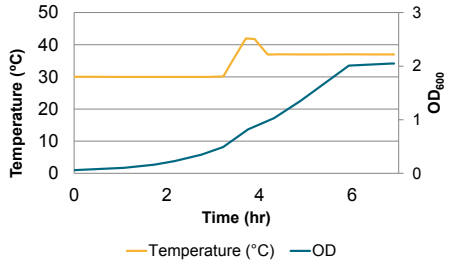

Figure 2. 10 L stainless steel fermentor: *E. coli* growth and culture temperature.

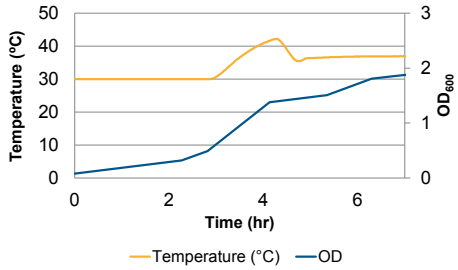

Figure 3. 300 L S.U.F.: *E. coli* growth and culture temperature.

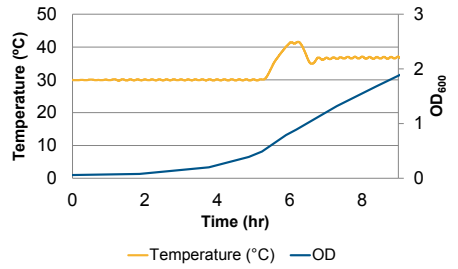

Figure 4. 30 L S.U.F.: *E. coli* growth and culture temperature.

Protein expression

Thermoinducible protein expression in the S.U.F. and stainless steel fermentors produced identical target protein accumulation patterns–total, soluble, and insoluble fractions (Figure 5).

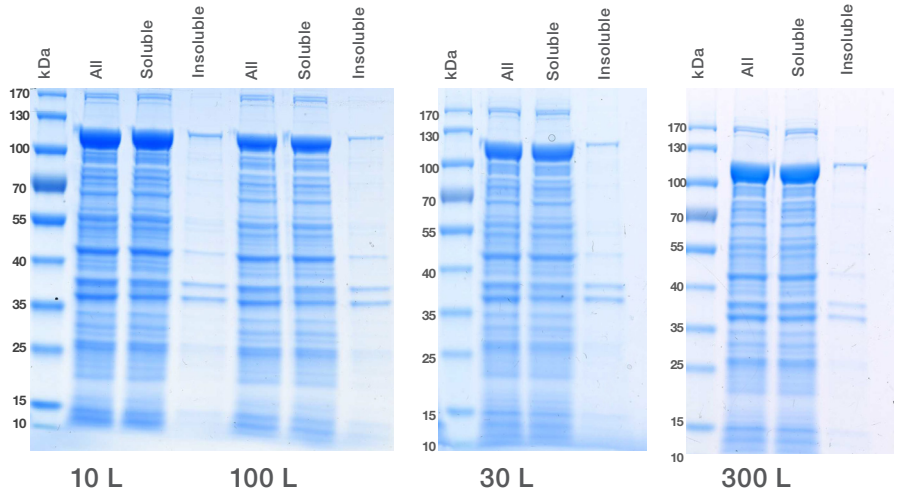

Figure 5. Protein fractionation on SDS-PAGE gels for each system.

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Biomass samples

Activity for DNA polymerase I was measured in million units per gram of wet weight and was normalized to millions of units per 1 g of biomass (Mu/g biomass) as shown in Figure 6. The detected activity in the negative control sample (biomass of *E. coli* expressing a different recombinant protein) represents the host DNA polymerase I activity which is naturally present in *E. coli* cells.

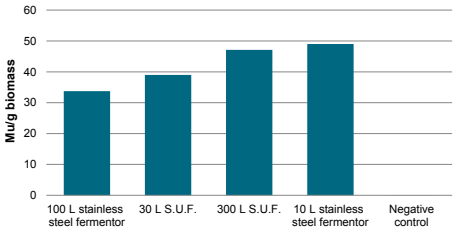

Figure 6. DNA polymerase I activity in biomass of *E. coli*.

Discussion

Time differences in heating were noted due to the methods used to heat each culture. The stainless steel fermentors used a heat exchanger directly with steam or process water. The 300 L S.U.F. system used an air-cooled TCU that had a 4,600 W heating rating with a hard-coded upper limit of 45°C. This was only rated at twice that of the 30 L 2,300 W TCU. For shorter heating times on the S.U.F. systems, it is recommended to use a TCU with a 24 kW heating rating for the 300 L, and for both the 30 L and 300 L sizes, a hard-coded upper limit of 55°C, or a heat exchanger system with valves to support heating and cooling solutions.

The final OD_{600} of the cultures did not differ significantly despite the difference in the heating time as indicated by Figures 1–4.

The recombinant DNA polymerase I yield obtained was similar in all cultures, i.e., >95% of polymerase was expressed as soluble protein fraction (Figure 5), with catalytic activity higher in single-use fermentors overall (Figure 6). Higher enzyme activities in the biomass obtained in the S.U.F. and 10 L stainless steel fermentor were most likely due to elongated heating times and thus more efficient induction of recombinant DNA polymerase I synthesis. The highly similar yields of the target enzyme revealed that the dynamic culturing conditions were maintained at favorable levels for the cellular metabolism and target product folding in both types of bioreactors.

The volume of the fermentors and the increased time during thermoinduction did not have any negative effect on the target enzyme yield—enabling an excellent scalability, flexibility of the process, and transferability from the stainless steel fermentors to the S.U.F.s.

Conclusion

Although the heat induction time was longer in the 300 L S.U.F., the product yield (soluble amount) (Figure 5) and catalytic activity (Figure 6) of the expressed enzyme were not significantly different. The target enzyme production process from the stainless steel fermentor was efficiently transferred to the 30 L S.U.F. and scaled to the 300 L single-use production fermentor without losing target protein yield and catalytic features.

References

1. Valdez-Cruz NA, Caspeta L, Pérez NO, et al. (2010) Production of recombinant proteins in *E. coli* by the heat inducible expression system based on the phage lambda pL and/or pR promoters. *Microbial Cell Factories* 9:18.
2. Möbius SS, Möbius TL (2012) *Handbook of liquid scintillation spectrometry.* Eggenstein-Leopoldshafen Karlsruher Inst. für Technologie. pp 1–115.

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